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PHOSPHORYLATIONS BY ADENOSINE 5'-TRIPHOSPHATE

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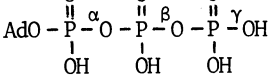
Abstract - The ^{31}P NMR chemical shifts of salts of adenosine 5'-triphosphate, $ATPH_2^{2-}$ $2(CH_3)_4N^+$ and $MgATPH_2$ have been measured in D_2O solutions at several pD values. The results are compared with those obtained with adenosine mono- and diphosphate salts. The effects exerted by magnesium on the ^{31}P NMR signals of nucleotides are related to changes in conformation and acid strength of the polynucleotide chain resulting from the binding of divalent metal cations to pyrophosphate oxyanions. The three possible bidentate complexes interconvert rapidly on the NMR time scale in solution at pH > 7. The effects of calcium and magnesium on the ^{31}P NMR signals of ATP are very similar. Rates of nonenzymatic hydrolysis of $ATPH_2^{2-}$ $2(CH_3)_4N^+$ and $MgATPH_2$ in aqueous and in acetonitrile-water solutions have been determined in the pH range 0-10 utilizing liquid chromatography as the analytical tool. Hydrolysis of the tetraanion, ATP^{4-}, and trianion, $ATPH^{3-}$, occurs by an elimination-addition mechanism via a metaphosphate intermediate, PO_3^-, in the absence and in the presence of magnesium. Without magnesium, the trianion generates metaphosphate faster than the tetraanion by factors of 18 and 12 in water and in acetonitrile-water, respectively. Magnesium ions have no significant effect on the rate of formation of metaphosphate from the trianion, but increase the rate of metaphosphate formation from tetraanion by factors of $\sim$ 10 and $\sim$ 60 in water and acetonitrile-water respectively. The hydrolysis of the monoanion, $ATPH_3^-$, and of the unionized acid, $ATPH_4$, takes place by an addition-elimination mechanism via an oxyphosphorane intermediate (pentacovalent phosphorus). Probably both types of mechanisms participate in the hydrolysis of the dianion, $ATPH_2^{2-}$. The formation of tert-butyl phosphate when tetra-n-butylammonium salts of ATP are allowed to react in tert-butyl alcohol solution is taken as an indication of metaphosphate formation in reactions of the tetraanion and the trianion. It is proposed that, in enzymatic P_γ phosphoryl transfer, the complex $MgATP^{2-}$ binds tightly to the active site by interactions of magnesium with amino acid residues, notably histidine, and by interaction of the polyphosphate chain, the ribose, and the adenine ring with other amino acid residues. The enzyme-bound $MgATP^{2-}$, with one electrostatic P_γ oxyanion-magnesium interaction, becomes protonated at P_γ by histidine ($HisH^+$) in the fully organized active site. The relatively electrophilic complex, AdOP(O)($\bar{O}$)O.P(O)($\bar{O}$)O.P(O)(OH)OMg$^+$, accepts the nucleophile at P_γ and forms the oxyphosphorane intermediate which collapses to the products, MgADP and phosphorylated-nucleophile, before or after undergoing permutational isomerizations. This hypothesis is applied to the catalysis of the hydrolysis of ATP by muscle myosin in the presence of magnesium or calcium ions, and to the energy transduction of muscle contraction, which occurs only in actomyosin MgATPase.

INTRODUCTION

This paper is concerned with several aspects of the chemistry of adenosine 5'-triphosphate (ATP),



(1) The coordination of magnesium ions with ATP in aqueous solution. We have investigated the effect of magnesium and calcium ions on the ^{31}P NMR signals of ATP, to ascertain if the spectral changes induced by divalent cations are associated with changes in polyphosphate chain conformation and acidity, or are due to more specific effects of the cations [1].

(2) The mechanism of nonenzymatic hydrolysis of ATP in aqueous solution and in a mixed acetonitrile-water medium, in the pH range from 0 to 10. We have searched for effects of magnesium ions on hydrolysis rates in these solvents, in order to establish a background for an analysis of the role of magnesium in the enzymatic reactions of ATP [2].

(3) The mechanisms of enzymatic phosphoryl transfer by ATP. From the existing literature on hydrolysis of MgATP and CaATP by skeletal muscle myosin in the presence and in the absence of the protein actin, a hypothesis for the mechanism of hydrolysis and for the ATP energy transduction coupled to muscle contraction has been developed [3-5].

COORDINATION OF MAGNESIUM WITH ATP IN SOLUTIONS

The significant effects of divalent metal ions on the ^{31}P NMR chemical shifts of ADP and ATP in aqueous solution have been known for some time [6-10]. It was assumed that the phosphorus NMR data provided unequivocal support for the existence in solution, of a complex with magnesium electrostatically bound to the Pβ and Pγ oxyanions of ATP. This view was later challenged [11], and it was suggested that the cation was bound exclusively to the nonterminal Pβ center of ATP. More recently, it was concluded that the ^{31}P NMR data do not specify the site occupied by magnesium in the polyphosphate chain in solution [12,13].

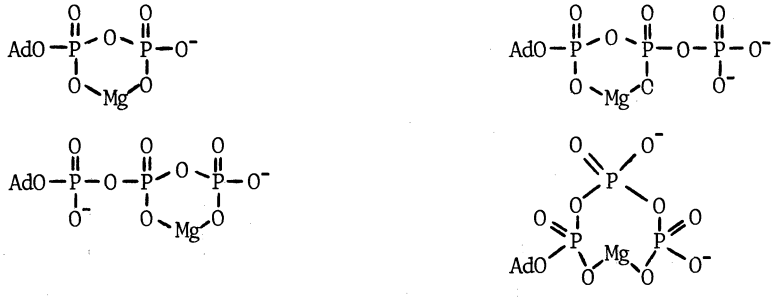
We investigated this problem utilizing carefully purified salts made from the acid, $ATPH_4$, namely: $ATPH_2^{2-}$ 2 Me_4N^+ and $MgATPH_2$. High resolution ^{31}P NMR spectra of 0.02 M D_2O solutions of the salts were obtained at 145.7 MHz at 22° C, at three pD values: 8.20, 6.20 and 3.70 (pD = pH + 0.4). The concentration of ATP in these solutions is kept low to minimize self-association of the nucleotide [14]. The purification procedure eliminates traces of paramagnetic metal ions. The study included other salts: $ADPH^{2-}$ 2 Me_4N^+ and MgADPH prepared by the same procedure, as well as salts from AMP and simpler phosphomonoesters for comparison purposes. The results are summarized in Figure 1.

The data in Fig. 1 must be analyzed in the context of some additional information. The replacement of tetramethylammonium cation by magnesium ions in solutions of the phenyl phosphate dianion, $ArOPO_3^{2-}$, has only a slight downfield effect on the ^{31}P NMR shift ($\Delta \sim 0.4$ ppm). The same insensitivity of the ^{31}P signal toward cation structure is noted in the dianion derived from adenosine 5'-monophosphate, AMP^{2-} ($\Delta \sim 0.4$ ppm, upfield). The ^{31}P shift is virtually the same in the presence of tetramethylammonium and of magnesium ion in the monoanions of phenyl phosphate, $ArOPO_3H^-$, and of $AMPH^-$. These data show that salt formation between magnesium and certain monophosphate oxyanions does not significantly affect the ^{31}P NMR chemical shift, relative to that of the ammonium salt.

Coordination of magnesium ions with ADP at pD 8.20 has the same effect on the Pα and Pβ centers, i.e. causes a 0.9 ppm downfield shift in both signals. At pD 8.20 the predominant species is the trianion, since the pKa_3 values are 6.9 for $ADPH^{2-}$ 2 Me_4N^+, and 5.3 for MgADPH, respectively.

Coordination of magnesium ions with ATP at pD 8.20 causes downfield shifts of the Pα and Pγ signals to the extent of 0.8 and 2.0 ppm, respectively. The nonterminal Pβ signal is the one most significantly affected by magnesium, and shows a downfield shift of 3.9 ppm. From data on ATP acidity, it follows that the predominant species at pD 8.20 is the tetraanion, ATP^{4-}.

The following Scheme shows the various possible bidentate complexes of magnesium ions with ADP and ATP. The most reasonable explanation for these effects by magnesium at pD 8.20 is to assume that in solution the three magnesium-ATP complexes are in equilibrium with each other, and that this equilibrium is relatively rapid in the time scale of NMR at 22° C. The changes in conformation of the polyphosphate chain as the various oxyanions become electrostatically bound to the divalent cation cause the changes observed in the chemical shifts of the three nuclei. The effect of magnesium on the signals does not correlate with the position of the metal in the chain. Moreover, calcium has virtually the same effect as magnesium on the three signals of ATP, under the same conditions [1].



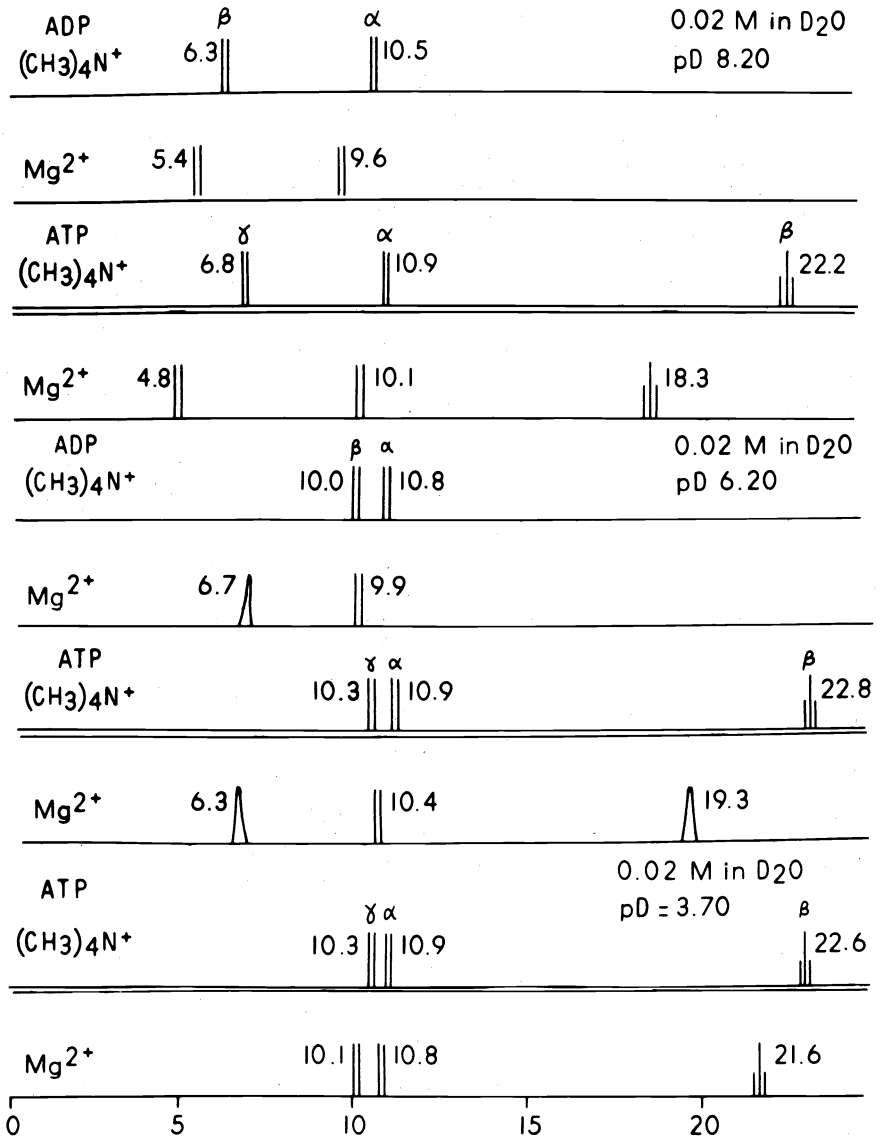


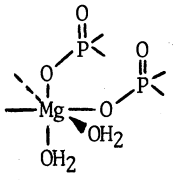
Fig. 1 Proton-decoupled ^{31}P NMR signals of adenosine 5'-diphosphate and triphosphate in 0.02 M D_2O, in the presence and in the absence of magnesium ions.

The spectra at pD 6.20 disclose that a moderate increase in acidity has little effect on the Pα signals of ADP and ATP, and only a slight upfield shift in the Pβ signal of ATP in the absence of magnesium. This is as expected, since the first protonation does not occur at any of these centers. The increase in acidity results in a substantial ($\Delta \sim 3.5$ ppm) upfield shift in the signals due to the terminal phosphorus centers in ADP and ATP, in the absence of magnesium. Again, this effect would be expected from Pβ and Pγ protonation in ADP and ATP, respectively. The addition of the first proton to the oxyanion of a doubly-charged phosphorus center is accompanied by a relatively large ($\Delta \sim 3$ ppm) upfield shift of the ^{31}P signal in both phenyl phosphate and AMP. Protonation of the second oxyanion does not have much effect on the shift ($\Delta < 0.5$ ppm).

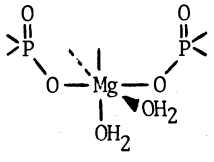
An interesting effect is observed on the Pβ signal of ADP, and on the Pγ and Pβ signals of ATP, when the acidity is increased to pD 6.20 in the presence of magnesium. These signals, but not the Pα signals, are broadened. This phenomenon could reflect a weakening of intramolecular bidentate magnesium-oxyanion binding at higher acidity, and the establishment of intermolecular binding: $>P(O)OMgOP(O)<$ with the cation always fixed at the Pα center.

At still higher acidity, pD 3.70, the ^{31}P shifts in the presence of magnesium approach the values observed in the absence of this cation, suggesting a disruption in binding of the metal ion to the chain.

X-ray diffraction analysis of aquo-magnesium phosphodiester complexes [15] shows that a phosphate oxyanion in the role of electrostatic ligand in the inner coordination sphere of octahedral magnesium ion gives rise to a short interatomic distance: Mg-O (phosphate), 2.04 Å, comparable to that of water as donor ligand, Mg-O (water), 2.15 Å. The magnesium-oxygen tight binding in phosphate esters is reflected in the relatively close O...O nonbonded interatomic distances when phosphate oxyanions occupy cis and trans positions in the magnesium octahedral inner coordination sphere:



Cis O...O, 2.91 Å

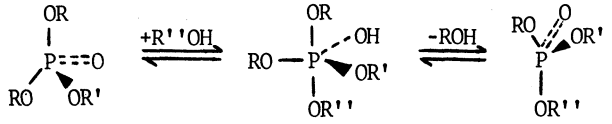


Trans O...O, 4.08 Å

In summary, the interatomic distance Mg-O (phosphate ester) in the crystalline state is quite short, and in this sense, this configuration represents "tight binding" of magnesium ions to oxyanions of phosphates and pyrophosphates. In aqueous solutions, tight binding in a magnesium phosphate and pyrophosphate ester complex implies short Mg-O distance but a relatively rapid, intramolecular and/or intermolecular exchange of closely bound ions in the ion-pair. The effect of the medium on interatomic distance and rate of exchange among members of this type of complex is not known, although presumably, changes of medium in the direction of lower dielectric constant and greater hydrophobicity should lead to tighter Mg-O (phosphate ester) binding in the sense of shorter interatomic distance and slower exchange rates.

MECHANISMS OF NONENZYMATIC NUCLEOPHILIC DISPLACEMENTS ON PHOSPHATE ESTERS

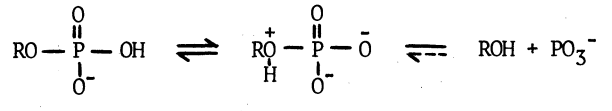
Nucleophilic displacements on phosphotriesters and phosphodiesters are satisfactorily described by an addition-elimination mechanism via the oxyphosphorane intermediate (R' = carbon or hydrogen) [16-21].



Phosphodiester anions are less reactive than their confugate acids [22]. This is a reasonable corollary of the oxyphosphorane mechanism, since an oxyanion ligand should decrease the electrophilicity of phosphorus, and the stability of the resulting oxyphosphorane, as well as of the transition state leading to the pentacovalent intermediate.

An oxyphosphorane intermediate that provides no direct or indirect information of its existence, for example from NMR studies or isotopic exchange experiments, is operationally indistinguishable from a transition state analogous to that postulated for S_N2 displacements at tetrahedral carbon. However, there is a growing body of evidence in support of the hypothesis that structures with pentavalent phosphorus are formed as intermediates with varying life-times in displacements on phosphate esters by the addition-elimination mechanism [23-27]. Hydroxyphosphoranes have been directly observed in solutions in equilibrium with phosphate esters and phenols by means of ^{31}P NMR spectroscopy [28,29].

Nucleophilic displacements on the nonionized form of phosphomonoesters, $ROPO_3H_2$, also proceed by the addition-elimination mechanism [30]. However, there is now ample evidence that the monoionized form of some (but not all) phosphomonoesters, reacts by an elimination-addition mechanism via the monomeric metaphosphate anion [21,31,32]:

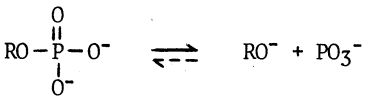


$$PO_3^- + R'OH \rightleftharpoons R'OPO_3H^-$$

The proton shift indicated in the monoanion reaction [31] seems to be required to provide the necessary driving force for the rate-limiting elimination step. The elimination of the

acid, PO_3H, in contrast to that of the anion, PO_3^-, does not appear to furnish the necessary driving force for elimination.

The diionized form of phosphomonoesters also react via elimination-addition [33-34]:



The metaphosphate anion has been observed in the gas phase by means of negative-ion mass spectrometry [35]. However, all the evidence for metaphosphate in solution is of an indirect nature at the present time [36,39]. An important property of the metaphosphate-ion is its extremely powerful electrophilicity [40]. The anion, PO_3^-, should react indiscriminately with any nucleophile present in the environment in which it is generated.

These mechanistic conclusions are supported by studies in the series of aryl phosphates: phenyl, 4-nitrophenyl-, and 2,4-dinitrophenyl phosphates in water, alcohol, acetonitrile, and mixtures of these solvents [38,39,41]. In phosphomonoesters, and in general, in compounds of type XPO_3H_2, the acid strength of the ionizable protons plays a critical role, since it determines the state of ionization, and hence the operative reaction mechanisms under a given set of conditions. The nature of the medium is also quite important in this respect. Acid strength of oxyanions decreases in the solvent series: H_2O > alcohols > acetonitrile. For a given compound, XPO_3H_2, under a given set of conditions, one expects a higher degree of protonation in acetonitrile than in water. This effect may be significant in enzymatic reactions of ATP when the reactive site is relatively hydrophobic. In the active site pocket, the tetraanion ATP^{4-} is probably a stronger base than in aqueous solution. This should favor proton transfer from protonated histidine residue in the protein: $ATP^{4-} + HisH^+ \rightleftharpoons ATPH^{3-} + His$. The conjugate acid of histidine (pKa $\sim$ 7 in water) is also weaker in hydrophobic media than in water, but this decrease in acidity with the medium is smaller for aminium acids than for oxyacids. Magnesium ions increase the acidity of protons in ATP. Hence, the acid-decreasing effect of hydrophobic media may be crucial in facilitating proton transfer from histidine to the magnesium complex of ATP in the enzyme active site: $MgATP^{2-} + HisH^+ \rightleftharpoons MgATPH^- + His$.

A second, and quite significant medium effect arises as follows. The ground state of phosphates is quite susceptible to stabilization through solvation. On the other hand, the transition state that leads to the less polar oxyphosphorane should be less susceptible to solvation stabilization. It follows that the rate of formation of an oxyphosphorane intermediate from a phosphate and a nucleophile increases in going from media of greater to lesser solvating power due to this differential ground state solvation. A similar argument can be developed in the elimination-addition mechanism, since the metaphosphate anion:

and the transition state for its formation should be less polar, and less susceptible to stabilization through solvation, than the ground state phosphate.

In summary, nucleophilic displacements on derivatives of phosphoric acid without and with ionizable protons are satisfactorily described by mechanisms which involve intermediates with five-coordinate or three-coordinate phosphorus, i.e. oxyphosphoranes or the monomeric metaphosphate anion. There is no need to postulate a spectrum of reactivity from limiting S_N2 - like to S_N1 - like transition states in these types of reactions, in the absence and in the presence of nucleophilic catalysts.

EFFECT OF MAGNESIUM IONS ON THE NONENZYMATIC HYDROLYSIS RATES OF ATP

Several qualitative [43-46] and quantitative [47-52] studies of ATP hydrolysis have been described. However, the mechanism of this reaction is not well understood [53].

We have carried out a study of ATP hydrolysis rates in solutions of two salts, $ATPH_2^{2-}$ $2(CH_3)_4N^+$ and $MgATPH_2$ in 0.01 M solution in water, and in 1 : 1 (v/v) acetonitrile-water, at 70° C. In these studies, the pH of the solution was kept constant within $\pm$ 0.05 pH units, and the measurements were extended from pH 0 to 10. Some of the results are given in Table 1. Additional experiments confirmed that the buffer components had no effect on these reaction rates.

The pH-rate profile for the hydrolysis of ATP in water is given in Figure 1. The curve in the absence of magnesium ions levels off at pH > 9.5. The corresponding pH-rate profile for the hydrolysis in acetonitrile-water is not reproduced; it differs from the profile in

water in two respects. (a) The nearly flat portion of the curve obtained in the absence of magnesium is displaced toward higher pH values due to the decrease in acidity of ATP in the mixed solvent relative to water. (b) The pH-rate profile in the presence of magnesium ion curves upwards above pH 6 due to an increase in hydrolysis rate in acetonitrile-water vs water that is caused by magnesium at the higher pH values (see below).

TABLE I. Rates of Hydrolysis of Adenosine 5'-Triphosphate at 70.0 ± 0.1° C in 0.01 M Solutions (μ = 0.2)[a]

pH	k, min^{-1}	$t_{1/2}$	pH	k, min^{-1}	$t_{1/2}$
Solvent: Water					
Cation:	$(CH_3)_4N^+$		Cation:	Mg^{2+}	
9.30	3.28×10^{-5}	352 hr	...	...	...
9.03	3.88×10^{-5}	298 hr	...	...	...
8.30	1.15×10^{-4}	100.5 hr	8.25	3.77×10^{-4}	30.6 hr
7.66	2.65×10^{-4}	43.6 hr	7.63	3.82×10^{-4}	30.2 hr
6.69	5.11×10^{-4}	22.6 hr	6.59	4.15×10^{-4}	27.8 hr
5.44	6.08×10^{-4}	19.0 hr	5.36	5.07×10^{-4}	22.8 hr
3.98	7.13×10^{-4}	16.2 hr	3.92	4.90×10^{-4}	23.6 hr
2.66	1.76×10^{-3}	6.6 hr	2.64	9.29×10^{-4}	12.4 hr
1.45	1.98×10^{-2}	35 min	1.52	1.66×10^{-2}	42 min
0.6	1.39×10^{-1}	5 min	...	...	...
Solvent: Acetonitrile - Water, 1 : 1 v/v					
Cation:	$(CH_3)_4N^+$		Cation:	Mg^{2+}	
9.50	3.79×10^{-5}	305 hr	...	...	...
8.30	2.16×10^{-4}	53.6 hr	8.14	2.23×10^{-3}	5.2 hr
7.85	2.81×10^{-4}	41.0 hr	...	...	...
6.70	3.21×10^{-4}	36.0 hr	6.66	2.39×10^{-3}	4.8 hr
4.75	4.50×10^{-4}	25.7 hr	4.65	7.60×10^{-4}	15.2 hr
3.39	9.77×10^{-4}	11.8 hr	3.28	5.36×10^{-4}	21.6 hr
2.40	3.08×10^{-3}	3.8 hr	2.10	1.58×10^{-3}	7.3 hr
1.25	3.93×10^{-2}	18 min	1.34	2.90×10^{-3}	75 min

[a] $ATPH_2^{2-}$ $2(CH_3)_4N^+$ and $MgATPH_2$ as starting materials. Buffer contained $(CH_3)_4N^+$ ions exclusively.

An analysis of these hydrolysis rate data must take into account the appropriate acid-base equilibria. In water, at pH < 2, the acid, $ATPH_4$, and its monoanion, are the only species present in significant amounts. In the pH range 2.0-7.0, the appropriate ratios of monoanion, dianion and trianion, must be taken into consideration in deciding the type of structure that can serve as substrate for the hydrolysis. In the pH range 7.0-9.0, one is concerned only with trianion and tetraanion. At pH > 9.0, the tetraanion is the only ionic species present. Since magnesium ions increase the acidity of the last ionizable proton of ATP to a value of 5.36 in water and 5.78 in acetonitrile-water, the tetraanion is practically the only potential substrate at pH > 7.0 and pH > 7.5 in these two solvents when the metal ion is present in the solutions.

The hydrolysis rates for these reactions were determined using a liquid chromatographic technique. The ATP and its hydrolysis products, ADP and AMP, were separated by HPLC on a Partisil SAX anion exchange column with the eluent monitored by a UV detector at 260 nm. The area under each peak was measured for a series of aliquots taken at appropriate times and the rate constants calculated by standard procedures [54].

The hydrolysis of ATP in 0.1 N HCl (pH = 1.5) occurs either by exclusive P_γ cleavage, or by a combination of P_γ cleavage and attack at P_β with displacement of P_i and formation of ADP. This conclusion follows from the observation that there is little AMP formed within the first half-life of hydrolysis in 0.1 N HCl. Under these conditions, the reaction: $H_2O + ATP \rightarrow ADP + P_i$ is about six times faster than the reaction: $H_2O + ADP \rightarrow AMP + P_i$, and formation of AMP by this reaction sequence does not complicate the analysis of the primary hydrolytic process. However, in 1N HCl, the two reactions: $H_2O + ATP \rightarrow ADP + P_i$ and $H_2O + ADP \rightarrow AMP + P_i$ proceed at about the same rates, and one cannot precisely define the primary ATP hydrolysis pathway. However, if there is P_α attack or P_β attack with displacement of AMP and formation of PP_i, these pathways contribute less than 10% to the overall hydrolysis in 1 N HCl, since one observes only about 10% of AMP within the first half-life of the reaction.

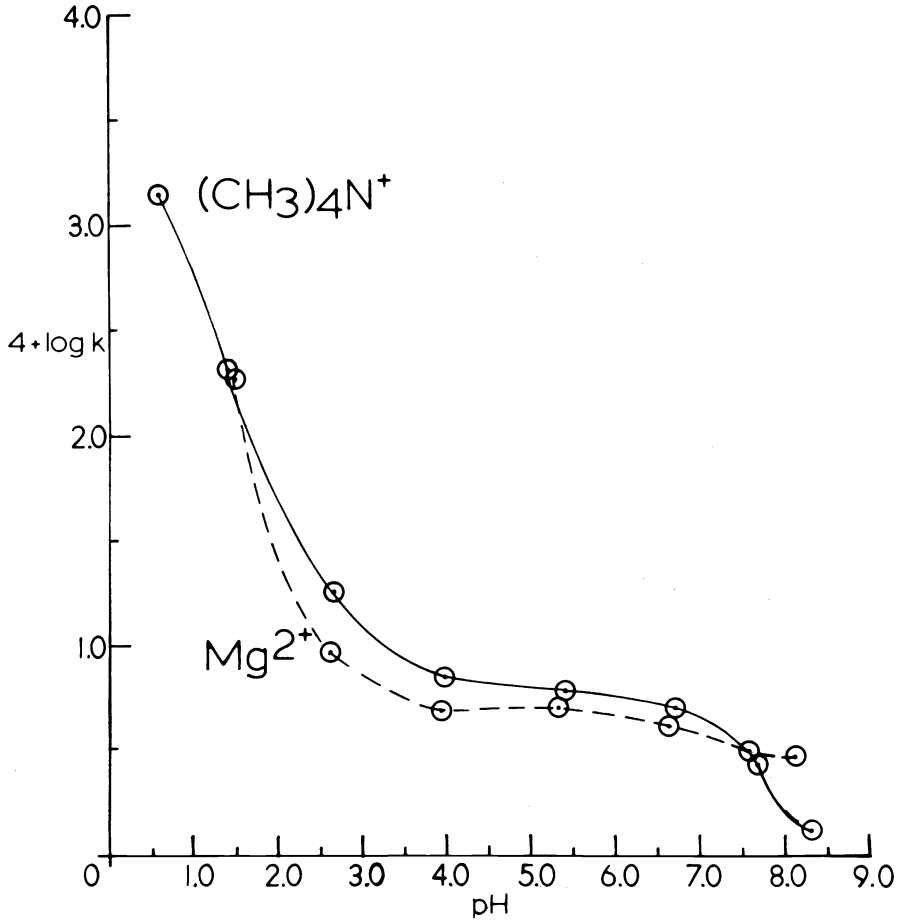
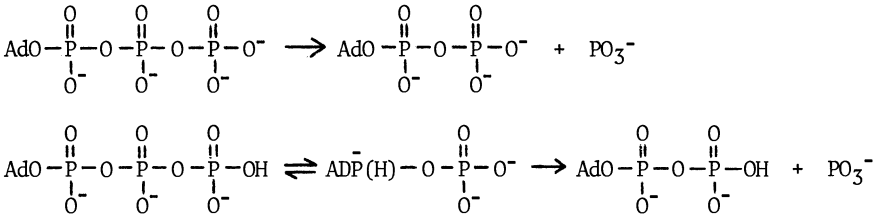


Fig. 2. pH-Rate profile for hydrolysis of adenosine 5'-triphosphate in 0.01 M aqueous solution at 70° C.

The isotopic labeling experiments that are needed to describe precisely the relative contributions of Pα, Pβ and Pγ cleavage in strongly acidic media during nonenzymatic hydrolysis of ATP have not been described. At pH > 5.0, hydrolytic cleavage occurs exclusively by Pγ cleavage, as suggested by the data presented below.

The most significant results of the investigation, and our interpretation of them are as follows.

The tetraanion, ATP^{4-}, and the trianion, $ATPH^{3-}$, which have a terminal Pγ center of the type XPO_3^{2-} and XPO_3H^-, respectively, undergo nucleophilic displacements at that center by an elimination-addition mechanism, in which formation of monomeric metaphosphate anion is rate limiting:



The highly electrophilic metaphosphate ion reacts rapidly with the nucleophile to yield the product: $ROH + PO_3^- \longrightarrow ROPO_3H^-$. The proton-shift assumed to occur in the trianion allows the elimination of the anion, rather than of its conjugate acid PO_3H, as was discussed in a previous section. This "metaphosphate hypothesis" for reactions of the ATP tetraanion and trianion has been previously considered [55]. Our hydrolysis data are indeed consistent with this interpretation, but this mechanism may be limited to hydrolysis of the nucleotide in the absence of enzymes.

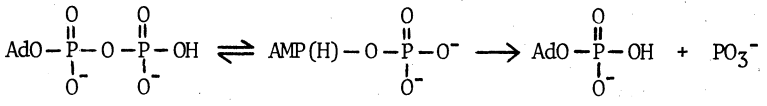
The formation of metaphosphate as an intermediate in the hydrolysis of the ATP tetraanion and trianion is supported by the formation of tert-butyl phosphate when solutions of tetra-n-butylammonium salts of these anions in mixtures of acetonitrile-tert-butyl alcohol are kept several hours at 70° C:

$$ATP^{4-} + (CH_3)_3COH \longrightarrow (CH_3)_3COPO_3^{2-} + ADPH^{2-}$$

$$ATPH^{3-} + (CH_3)_3COH \longrightarrow (CH_3)_3COPO_3H^{-} + ADPH^{2-}$$

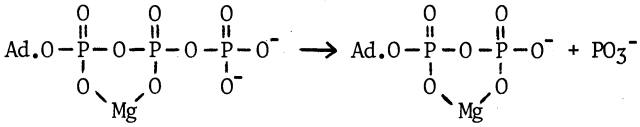
It is unlikely that tert-butyl phosphate can be derived from any reaction other than the entrapment of the highly reactive metaphosphate intermediate. The rate of decrease of ATP concentration is insensitive to the structure of the alcohol, and isopropyl alcohol yields isopropyl phosphate at a comparable rate.

It is assumed that the reaction of the $ADPH^{2-}$ dianion with tert-butyl alcohol, as well as the hydrolysis of this dianion, occur also by an elimination-addition mechanism:



Reaction of the metaphosphate anion with alcohol or water, would then produce the final products: $AMPH^-$ monoanion plus monoalkyl phosphate or P_i.

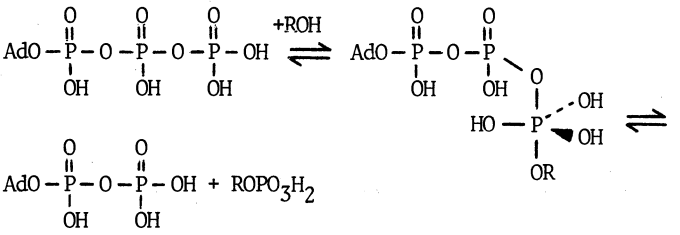
Returning now to the rates of hydrolysis given in Table 1. In the absence of magnesium ion, the rate of formation of metaphosphate from the ATP^{4-} tetraanion is slightly (∿ 15% faster) in the mixed solvent vs water. The trianion generates metaphosphate faster than the tetraanion by factors of 18 and 12 in water and in acetonitrile-water, respectively. The presence of magnesium ions in the solution does not significantly affect the rate of formation of metaphosphate from the trianion, $MgATPH^-$, in either of the two solvents. However, magnesium increases the rate of formation of metaphosphate from the tetraanion, $MgATP^{2-}$, by factors of 10 and 60 in water and in acetonitrile-water, respectively. This is understandable, if, as discussed in a previous section, solutions of $MgATP^{2-}$ contain three bidentate complexes of the cation and the polyphosphate chain in relatively rapid equilibrium with each other. The formation of metaphosphate and of $MgADP^-$ is faster from one of these complexes, since the divalent cation renders ADP^{3-} more nucleofugic by neutralizing two of the negative charges [56,57]. The more hydrophobic the medium, the tighter the complex and the faster the elimination step.



The hydrolysis of the ATP^{4-} tetraanion in water, in the absence of magnesium ions, is not significantly affected by the addition of either 10 or 50 molequivalents of quinuclidine, pyridine or imidazole, in 0.01 M solutions at 70° C. This is in agreement with our finding that these amines do not increase the rate of nucleophilic displacements on aryl phosphate dianions, $AROPO_3^{2-}$ [39,41]. As discussed elsewhere, quinuclidine (although not pyridine or imidazole) exerts a powerful nucleophilic catalysis on displacements on certain aryl phosphate monoanions, $ArOPO_3H^-$. This phenomenon is best observed in acetonitrile solution [39,41].

The magnesium complex of the trianion, $MgATPH^-$, generates metaphosphate at about the same rate as the metal-free trianion, $ATPH^{3-}$. This is expected from the observed effects of magnesium ions on ^{31}P NMR signals of ATP at various pH values (cf. previous Section). One can say that protonation at P_γ weakens the binding of magnesium to the polyphosphate chain and hence disfavors MgADPH nucleofugicity, or that the $(MgP_\alpha P_\beta)ATPH^-$ complex disfavors the proton shift needed for metaphosphate formation.

The hydrolysis of the acid $ATPH_4$, and of the monoanion, $ATPH_3^-$, is assumed to occur by an addition-elimination mechanism and to involve an oxyphosphorane intermediate. This hypothesis is consistent with studies on the aryl phosphates. The strongly acidic conditions where the hydrolysis of ATP becomes relatively rapid insure the presence of significant amounts of species with a diprotonated terminal P_γ center, and also provides opportunity for acid catalysis of oxyphosphorane formation. Note that the rates differ by a factor of about 4200 at the extremes of the pH range 0-9.5 in water. As expected magnesium ions have little effect on hydrolysis rates at pH < 4.



An area of uncertainty remains, namely, whether the dianion, $ATPH_2^{2-}$, reacts via metaphosphate or via oxyphosphorane intermediates. Probably, both mechanisms are competitive in view of the possibility of proton shifts in solution (as contrasted to enzymatic reactions, where these proton shifts are restricted, see below).

One of the most interesting questions concerning the mechanisms of ATP hydrolysis under enzymatic and nonenzymatic conditions is whether the monoprotonated $P\gamma$ center of the trianion, $ATPH^{3-}$ is susceptible to nucleophilic catalysis by imidazole and by unhindered amines, in general. We have not been able to demonstrate this effect in solutions, even utilizing the powerful nucleophile quinuclidine. However, it is conceivable that the $P\gamma$ center of $ATPH^{3-}$ in the active site of an enzyme is electrophilic enough to accept an imidazole group from a histidine residue, or a carboxylate group from an aspartate or glutamate residues, or a hydroxyl group from a serine or tyrosine residue to form oxyphosphorane intermediates, and then a phosphorylated enzyme intermediate. An attractive hypothesis is that one of the main roles of the enzyme in nucleophilic displacements on MgATP is to fix the electrostatic binding of magnesium ions at a $P\gamma$ oxyanion, and to direct the protonation of the remaining $P\gamma$ oxyanion by a protonated catalytic histidine residue. The $P\gamma$ center could then be electrophilic enough to accept water or other nucleophilic substrates, or nucleophilic amino acid residues from the enzyme.

CLASSIFICATION OF ENZYMATIC REACTIONS OF ATP AT PHOSPHORUS CENTERS

Regardless of mechanism, reactions of nucleophiles with ATP can proceed according to the following scheme, where X = nucleophile, PP_i = inorganic pyrophosphate, and P_i = inorganic phosphate.

$$P\alpha\ ;\ X + ATP \rightleftharpoons PP_i + AMP\text{-}X$$

$$P\beta;\ \begin{cases} X + ATP \rightleftharpoons P_i + ADP\text{-}X \\ X + ATP \rightleftharpoons AMP + PP_i\text{-}X \end{cases}$$

$$P\gamma\ ;\ X + ATP \rightleftharpoons ADP + P_i\text{-}X$$

In enzymatic reactions of ATP and other nucleoside 5'-triphosphates, the nucleophiles range from water to alcohols, enols, carboxylic acids, phosphomonoesters and certain amines. The nucleotidyl transferases direct attack at the $P\alpha$ center. These enzymes are wide spread, e.g. the amino acid-nucleotidyl transferases involved in amino acyl - tRNA synthestases [58]. The pyrophosphoryl transferases direct attack at the $P\beta$ center and are selective in the sense of causing the nucleofugicity of AMP (rather than of P_i). These enzymes are very rare, and apparently only three of them have been characterized: thiamine pyrophosphokinase, phosphoribosylpyrophosphate synthetase, and 7,8-dihydro-2-amine-4-hydroxymethyl-pteridine pyrophosphokinase [59]. The phosphoryl transferases direct attack at the $P\gamma$ center, and are represented by few ATPases (water as nucleophile) and many kinases [60].

There is, at present, no satisfactory theory to account for the factors which direct nucleophiles to the $P\alpha$, $P\beta$ and $P\gamma$ centers of ATP, and for the crucial role played by magnesium ions in these enzymatic displacements. The following section discusses one of these reactions types, namely the transfer of the phosphoryl group to water under catalysis by myosin.

ENZYMATIC HYDROLYSIS OF MgATP. ACTOMYOSIN MgATPase IN MUSCLE CONTRACTION

The central problem in enzyme-catalyzed ATP reactions, namely the recognition of the factors which direct nucleophiles toward the $P\alpha$, $P\beta$ or $P\gamma$ centers of ATP, has not been solved. Moreover, there are serious questions concerning the detailed chemical mechanism that operates during enzymatic displacements at the $P\gamma$ center of the nucleotide [61,62]. The same question does not arise in enzymatic displacements at the $P\alpha$ and $P\beta$ centers which possess only one ionizable proton. These reactions presumably take place by the same mechanism that operates in nonenzymatic displacements at those centers, i.e. addition-elimination via oxyphosphoranes.

A mechanism which brings the behavior of the P_γ center of ATP in line with that of the P_α and P_β centers involves the addition of the nucleophile to a complex of magnesium ion with a P_γ-protonated trianion: $AdOP(O)(\bar{O})O.P(O)(\bar{O})O.P(O)(OH)OMg^+$. This $MgATPH^-$ complex may have an eight-membered ring, $(MgP_\alpha P_\gamma)ATPH^-$ or may contain only one Mg-to-oxygen electrostatic coordination. However, the significant feature of the complex is that it is intimately bound to the protein, and becomes even more integrated with the enzyme during the actual operation of the active site. The interactions between the $MgATPH^-$ complex and the enzyme involves, in this hypothesis, donor coordination between histidine residues and the magnesium ion; as well as indirect binding of magnesium to other amino acid residues through metal-ligated water. These interactions reinforce other attachments of the nucleotide to the enzyme by ion-pairing of polyphosphate oxyanions with positively charged amino acid residues, by hydrogen bonding of the ribose to the protein, by intercalation of the adenine ring into the protein, and by hydrogen bonding of certain amino acids, e.g. aspartate or glutamate, to the C6-NH_2 group of adenine.

In the ATPases, addition of the nucleophile water, perhaps from its location in the coordination sphere of magnesium, to the $MgATPH^-$ complex tightly bound to the enzyme is assumed to generate an intermediate with pentavalent phosphorus. This oxyphosphorane is also intimately bound to the enzyme, and its collapse generates a "protein-product complex" formulated as enzyme-ADP.Mg.P_i, where the energy derived from the hydrolytic cleavage of ATP appears as relatively localized protein conformational strain. In general, in other phosphoryl transferases, the addition of the nucleophile to the $MgATPH^-$ complex is assumed to generate oxyphosphorane intermediates, although thermodynamic considerations will vary relative to those pertaining to the ATPases.

The original suggestion [3-5] of the formation of a "long-lived protein-product complex", enzyme-ADP.Mg.P_i, was based on the finding [15] that a magnesium cation is able to hold tightly together a pair of phosphodiester oxyanions in the crystal of a complex where the magnesium has octahedral hexacoordination. A related concept has subsequently been introduced to account for some properties of other enzymatic reactions [63].

In enzymatic reactions of ATP one is concerned not only with the mechanism of the particular nucleophilic displacement that is catalyzed by the enzyme, but also with the overall biochemical pathway that leads from the binding of MgATP to the final physiological or metabolic consequence of the process. In this section we consider in detail the problem of muscle contraction coupled to the hydrolysis of MgATP by the enzyme myosin, i.e., the problem of the energy transduction which takes place during actomyosin MgATPase activity.

Muscle contraction is basically a two-stage phenomenon in which the hydrolysis of MgATP catalyzed by myosin provides energy which is coupled to the contraction by interactions between myosin and actin [64-70]. The location of the ATPase active site, and the mode of interaction of myosin with actin are controversial subjects. It is known that the modification of two sulfhydryl groups, SH1 and SH2, in myosin by reaction with alkylating agents affects significantly the ATPase activity [71-73], and that the reactivity of SH1 and SH2 toward alkylating agents differs in the presence and in the absence of bound MgATP [74-76]. The SH1 and SH2 groups have been identified with cysteine 21 and cysteine 11, respectively, in a 92 amino acid fragment (P_{10} peptide) derived from the myosin globular head [77]. The P_{10} peptide has been located near the neck (or hinge) in the primary structure of myosin [78]. As shown in Fig. 3, the hinge is the junction of the head with the thin helical body of the molecule, and this junction plays a key role in contraction. Native myosin has two enzymatically active globular heads per molecule, and it is not known whether the two heads participate in the overall process in exactly the same manner.

The traditional view in muscle contraction has been that the myosin active site is situated at the end of the head opposite the hinge, i.e., at a site in the head distal from the hinge [64-70]. In these models, the hinge end of the head faces the backbone of the myosin filament where it cannot reach actin. Recently it was proposed that the active site of myosin is in the hinge region; and that localized conformational changes at the active site induced by enzymatic events are directly coupled to physical movements of the nearly flexible hinge region [79,80]. The hypothesis that the myosin active site is in the P_{10} fragment, i.e., at a site in the head proximal to the hinge, was subsequently advanced in conjunction with a chemical mechanism for the myosin-catalyzed hydrolysis of MgATP. This hypothesis addressed itself also to the mechanism of the actin-activation of the hydrolysis, and of the energy transduction involved in muscle contraction [3-5].

In the new picture of muscle contraction [5], the flexible hinge region of myosin can project out toward the actin filament at the stage where actin activates the hydrolysis of MgATP, as shown in the lower drawings of Fig. 4. Beginning at Fig. 4(a), the flexible hinge region is relaxed, i.e. in a low energy state with the active site unoccupied. The two proteins are firmly attached to each other by the "distal grip". In Fig. 4(b), MgATP is bound to the protein, and the active site of the ATPase is being created through a process of induced fit. The resulting configurational changes at the active site cause more and

more strain at the distal grip, which eventually causes actin to dissociate from myosin, as shown in Fig. 4(c). At this stage, the system as a whole [A + M*.MgATP] and the bound MgATP retain virtually all of their original energy content. However, a major redistribution of energy has occurred in which actin has gained conformational energy and myosin has lost a corresponding amount. This energy transfer between the two proteins, which should not be confused with the primary transduction process, will be reversed in a later step when actin and myosin recombine at the distal grip.

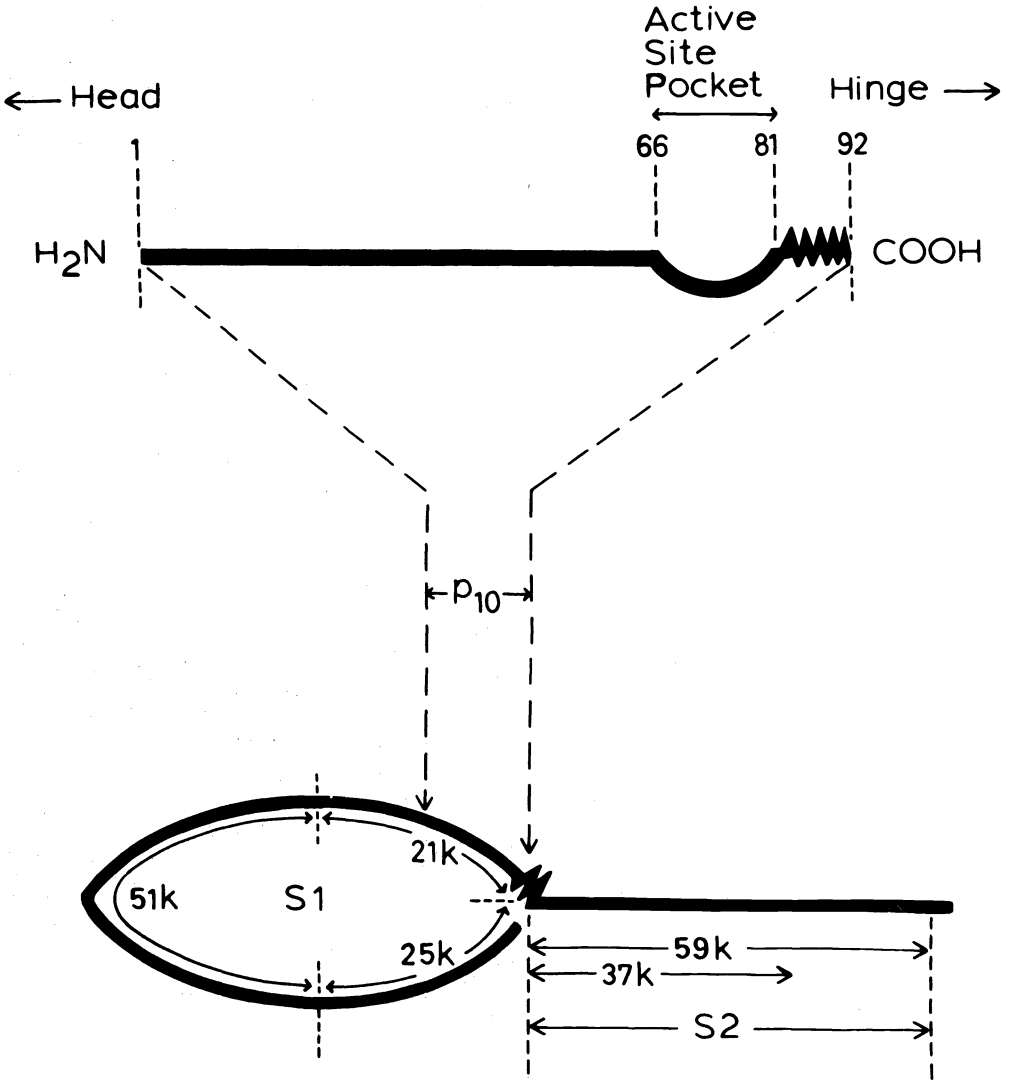
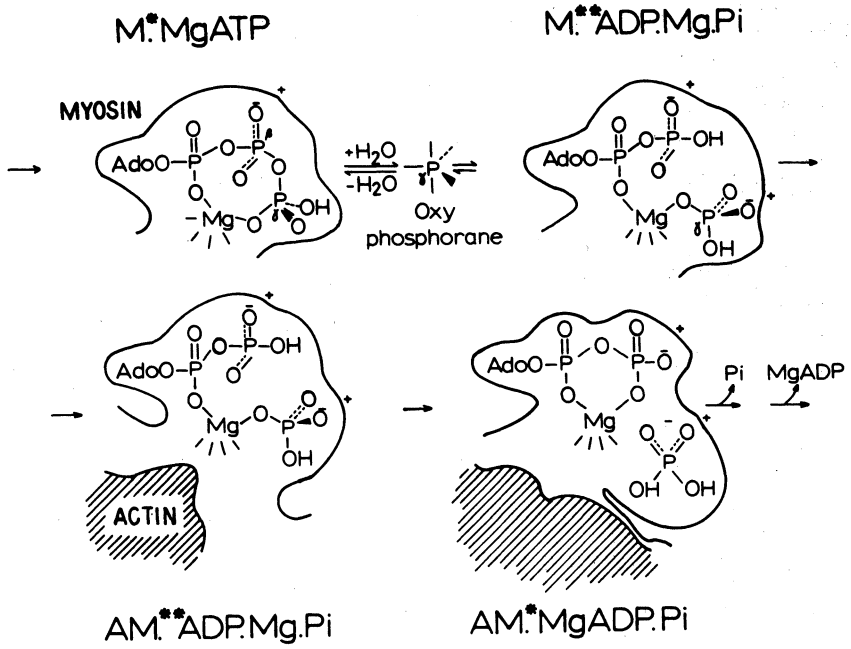


Fig. 3. Schematic representation of one of the two enzymatically active globular heads (subfragment S1) in the myosin molecule. The hinge connects the head to the thin helical body or subfragment S2. P_{10} is a 92 amino acid peptide situated in a 21,000 Daltons fragment of the molecule. The numbers along the enlarged P_{10} fragment (upper drawing) designate amino acid positions. Reproduced from Ref. 4 with permission of the publisher.

The primary energy transduction process is reversible and takes place during the transition: A + M^*MgATP to A + M^{**}.ADP.Mg.P_i. In the transduction, illustrated by Figs. 4(c) $\rightleftharpoons$ 4(d) $\rightleftharpoons$ 4(e), energy released by cleavage of the Pβ-Pγ bond of the bound MgATP is trapped in the form of localized conformational strain in the hinge region. The effect of the transduction is to bend the neck of the myosin molecule and markedly change the angle at the S1-S2 hinge; cf. Fig. 4(e). At this point an energy trap is set, as the bent conformation of the neck, and the active site is in a position that allows actin to spring the trap as it activates the next step in the enzymatic pathway. "Activation by actin" means an increase in the rate of release to the medium of the P_i that is formed as a result of the myosin-catalyzed MgATP hydrolysis. The release of P_i to the medium, not the MgATP hydrolysis, is

rate-limiting in the absence of actin.

Fig. 4(f) shows the interaction of actin at the active-site, which frees the distorted high-energy hinge region to move through lower energy states. Rotation of the head is the necessary consequence of the relaxion of the hinge. As the head rotates, the distal end moves onto the actin filament and binds to it establishing the distal grip; Fig. 1(g). Note that at this stage myosin engages actin weakly at the active site-hinge region, where the trap is sprung, and tightly at the opposite end of the head through the protein-protein interactions of the distal grip.



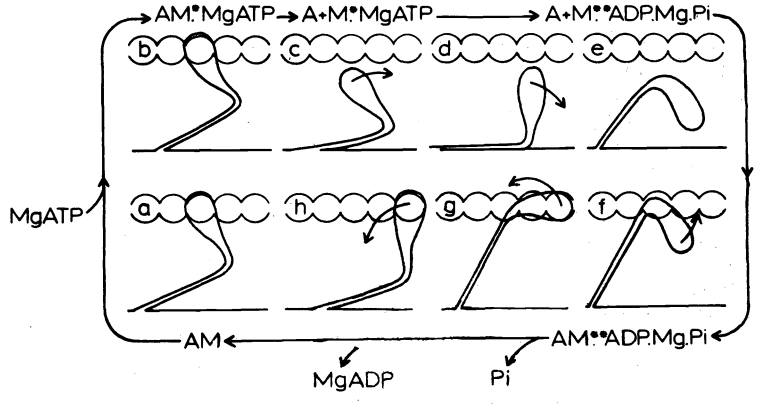


Fig. 4. Upper drawing: postulated structures for key intermediates in the pathway of MgATP hydrolysis by actomyosin. Lower drawing: postulated positions of the myosin head during the cycle of contraction. Reproduced from Ref. 5 with permission of the publisher.

Fig. 4(h) shows actin engaged to myosin only at the distal grip, and disengaged at the active site-hinge region. The head continues to rotate with the distal grip intact. As this happens, the release of conformational energy is coupled to the pull of the actin filament, which can be used for mechanical work. The translation of the actin filament relative

to myosin occurs during the pulling phase of the cycle, Figs. 4(g) → 4(h) → 4(a), when products of the enzyme hydrolysis are released, first P_i and then MgADP.

In this model of muscle contraction, the actin monomer that springs the trap (i.e. interacts at the active site) is different from the one that subsequently takes part in the distal grip. It takes at least two actin monomer, each approximately 50 Å in diameter, to span the 100 Å or more that separate the two ends of the myosin head. The conformation and angle changes at the hinge are the primary drive and are induced locally by nearby enzymatic activity. These movements of the hinge lead to the distal interactions with actin. The energy of MgATP is retained during the binding process and is released later when bound MgATP is hydrolytically cleaved. The loss of energy that is apparent in the relatively low energy content of M^*MgATP compared to M + MgATP is suffered by myosin, not by MgATP. The present model requires that the angle at the hinge changes with the state of the myosin active site even in the absence of actin. The energy of the distal grip is not used for work. Work is done at the expense of the free energy from the hydrolysis of one molecule of MgATP which is transferred to the hinge region where it exists as localized conformational strain. The conformational strain is released into a spatially directed mechanical motion able to do work.

In previous models of muscle contraction, both actin-activation and the pull of contraction depend on interactions between actin and the distal end of myosin. These distal interactions are the primary drive, indirectly changing the angle or conformation at a distant, virtually passive S1-S2 hinge. With a passive S1-S2 hinge there should be no preferred angles for the hinge in the various myosin-nucleotide intermediates in the absence of actin. In previous thermodynamic considerations of the contraction cycle, it is often assumed that all the useful energy of MgATP is transferred to the protein when the nucleotide binds to the active site of myosin. In those models the useful energy of MgATP is quantitatively conserved in the dissociation of actin from myosin; just that exact amount of energy must somehow be recovered for doing work when the two proteins recombine.

Finally, in the present, as well as in the previous models, the angle between the cross-bridge and the core of the myosin filament changes passively to maintain an approximately constant interfilament distance during the cycle. When the angle of the S1-S2 hinge changes actively in the new model, the angle of the cross-bridge adjusts in a way that obviates any lifting of the actin filament during the roll of the myosin head.

The upper half of Fig. 4 depicts key chemical intermediates proposed to account for the hydrolysis of MgATP, and for the actin-activation of the release of P_i to the medium after the hydrolytic step. It should be emphasized that the strain at the hinge region is imposed by the specific low-energy configuration of the magnesium ion and the bound products at the active site, represented by the M^{**}ADP.Mg.P_i complex. The tight chemical interactions between the magnesium, the phosphates and the active site in this intermediate serve to force and to hold the neighboring protein of the hinge region in a bent and highly strained position. The transduction step is reversible and there is an occasional return of M^{**}ADP.Mg.P_i to M^*MgATP. When this happens, the hinge relaxes but the whole system loses no energy. When actin interacts directly with the long-lived intermediate M^{**}ADP.Mg.P_i, the new complex M^*MgADP.P_i is formed; now, P_i is ready to be released to the medium.

One of the most interesting properties of myosin is its ability to utilize both MgATP and CaATP for hydrolysis in physiologically different ways. Myosin MgATPase couples with muscle contraction, myosin CaATPase does not, both in the presence of actin. In the absence of actin, the P_i that is formed in the hydrolysis of ATP is released to the medium at a much faster rate when the substrate for myosin is CaATP than when it is MgATP. As stated above, actin increases this rate of P_i release to the medium in myosin MgATPase, and the rate increase depends on actin concentration. These differences need not reside in differences in the chemical mechanisms by which MgATP and CaATP undergo hydrolysis, but rather in differences associated with the configuration of the M^{**}.ADP.Mg.P_i vs M^{**}.ADP.Ca.P_i. These differences, in turn could affect the mode of interaction of actin with the respective protein-product complexes derived from magnesium and from calcium. It may, in fact, be that the complex M^{**}.ADP.Ca.P_i does not represent an energy trap in the sense that the complex M^{**}.ADP.Mg.P_i does. In this case, the P_i generated in the hydrolysis of CaATP is released to the medium as fast as it is being formed.

It has been known for sometime that, in the absence of actin, the myosin-catalyzed hydrolysis of CaATP in a medium containing water enriched with $H_2{}^{18}\text{Ø}$ generates P_i containing only one labeled oxygen. $\text{AdOP(O)(Ō)O.P(O)(Ō)O.P(O)(Ō)OCa}^+ + H_2\text{Ø} \rightarrow \text{CaADP}^- + H_2PO_3\text{Ø}^-$. On the other hand, in the presence of actin, the myosin-catalyzed hydrolysis of MgATP in the same $H_2{}^{18}\text{Ø}$ enriched medium produces P_i containing more than one labeled water [81,82]. The conclusion derived from these observations has been that in the myosin MgATPase, but not in the myosin CaATPase, there is an extensive oxygen exchange between water and the P_γ center of intermediates M^*.ATP and M^{**}.ADP.P_i [4]. It has also been suggested that the oxygen exchange occurs in the oxyphosphorane intermediate that presumably separates the other two

intermediates. In one of these proposals, the permutational isomerication of the oxygens bound to trigonal bipyramidal phosphorus is said to occur by the "pseudorotation" mechanism [83], while in the other proposal the isomerization is assumed to take place by the "turnstile rotation" mechanism [4].

The actual number of isotopically labeled oxygen atoms from $H_2{}^{18}O$ that appear in the P_i released to the medium when the MgATP hydrolysis is carried out with native myosin and with proteolytic fragments derived from native myosin is not known with certainty. The reason for the uncertainty lies in difficulties inherent in the reproducibility of the proteolytic fragments, as well as the methods used for the isotopic analysis. The data thus far reported represents an average of the isotopic composition of the various possible P_i species, $H_2PO_3O^-$, $H_2PO_2O_2^-$, $H_2PO O_3^-$ and $H_2PO_4^-$, which correspond to no exchange, or to one, two and three exchanges, respectively. For this reason, and until the actual distribution of these isotopically labeled species of P_i is known with precision, no further discussion of the effect of proteolytic cleavage of myosin on the intermediate oxygen exchange is warranted. It is apparent, however, that the lack of oxygen exchange in CaATPase, and the extensive oxygen exchange in MgATPase are related to the respective rates of release of P_i to the medium. This, in turn, relates to the properties and the integrity of the corresponding protein-product complexes, $M^{**}.ADP.Ca.P_i$ and $M^{**}.ADP.Mg.P_i$.

In summary, much has been learned recently concerning the coupling of energy release during myosin ATP hydrolysis to the mechanical work of muscle contraction. Refinements of the techniques for the preparation of the proteolytic myosin fragments, and of isotopic analysis of the released P_i, in conjunction with studies of various divalent metal myosin ATPases, should give further insight into the link between chemical hydrolysis and physiological response in muscle contraction.

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REFERENCES

1. F. Ramirez and J. F. Marecek, Biochim. Biophys. Acta 589, 21 (1980).
2. F. Ramirez and J. F. Marecek, J. Am. Chem. Soc., Submitted for Publication.
3. F. Ramirez, K. K. Shukla and H. M. Levy, J. Theor. Biol. 76, 351 (1979).
4. K. K. Shukla, F. Ramirez, J. F. Marecek and H. M. Levy, J. Theor. Biol. 76, 359 (1979).
5. H. M. Levy, F. Ramirez and K. K. Shukla, J. Theor. Biol. 81, 327 (1979).
6. M. Cohn and T. R. Hughes, Jr., J. Biol. Chem. 237, 176 (1962).
7. H. Sternlicht, R. G. Shulman and E. W. Anderson, J. Chem. Phys. 43, 3123 (1965).
8. R. J. Labotka, T. Glonek and T. C. Myers, J. Am. Chem. Soc. 98, 3699 (1976).
9. C. T. Burt, T. Glonek and M. Barany, J. Biol. Chem. 251, 2584 (1976).
10. S. Tran-Dinh and J. M. Neumann, Nucleic Acids Res. 4, 397 (1977).
11. S. Tran-Dinh, M. Roux and M. Ellenberger, Nucleic Acids Res. 2, 1101 (1975).
12. E. K. Jaffe and M. Cohn, Biochemistry 17, 652 (1978).
13. E. K. Jaffe and M. Cohn, J. Biol. Chem. 254, 10839 (1979).
14. Y. F. Lam and G. Kotowycz, Can. J. Chem. 55, 3620 (1977).
15. P. Narayanan, F. Ramirez, T. McCaffrey, Y. F. Chaw and J. F. Marecek, J. Org. Chem. 43, 24 (1978).
16. W. E. McEwen and K. D. Berlin, Ed. Organophosphorus Stereochemistry, I, II, Dowden, Hutchinson and Ross, Stroudsburg, Pa. (1975).
17. F. Ramirez, B. Hansen and N. B. Desai, J. Am. Chem. Soc. 84, 4588 (1962).
18. F. Ramirez, O. P. Madan, N. B. Desay, S. Meyerson and E. M. Banas, J. Am. Chem. Soc. 85, 2681 (1963).
19. F. H. Westheimer, Acc. Chem. Res. 1, 70 (1968).
20. P. Gillespie, F. Ramirez, I. Ugi and D. Marquarding, Angew. Chem. Int. Ed. Engl. 12, 91 (1973).
21. F. Ramirez and J. F. Marecek, Pure & Appl. Chem., In Press.
22. F. Ramirez, J. F. Marecek, H. Tsuboi and Y. F. Chaw, Phosphorus & Sulfur 4, 325 (1978).
23. A. Munoz, B. Garrigues and M. Koenig, J. Chem. Soc., Chem. Comm., 219 (1978).
24. I. Granoth and J. C. Martin, J. Am. Chem. Soc. 100, 5229 (1978).
25. I. Granoth and J. C. Martin, J. Am. Chem. Soc. 100, 7434 (1978).
26. G. Kemp and S. Trippett, J. C. S. Perkin I, 879 (1979).
27. I. Sigal and F. H. Westheimer, J. Am. Chem. Soc. 101, 752 (1979).
28. F. Ramirez, M. Nowakowski and J. F. Marecek, J. Am. Chem. Soc. 99, 4515 (1977).
29. R. Sarma, F. Ramirez, B. McKeever, M. Nowakowski and J. F. Marecek, J. Am. Chem. Soc. 100, 5391 (1978).
30. C. A. Bunton, E. J. Fendler, E. Humeres and K.-U. Yang, J. Org. Chem. 32, 2806 (1967).
31. W. W. Butcher and F. H. Westheimer, J. Am. Chem. Soc. 77, 2420 (1955).
32. C. A. Bunton, Acc. Chem. Res. 3, 257 (1970).
33. A. J. Kirby and A. G. Varvoglis, J. Am. Chem. Soc. 88, 1823 (1966).
34. A. J. Kirby and A. G. Varvoglis, J. Am. Chem. Soc. 89, 415 (1967).

35. D. J. Harvan, J. R. Hass, K. L. Busch, M. M. Bursey, F. Ramirez and S. Meyerson, J. Am. Chem. Soc. 101, 7409 (1979).
36. C. H. Clapp and F. H. Westheimer, J. Am. Chem. Soc. 96, 6710 (1974).
37. J. Rebek, F. Gavina and C. Navarro, J. Am. Chem. Soc. 100, 8113 (1978).
38. F. Ramirez and J. F. Marecek, J. Am. Chem. Soc. 101, 1460 (1979).
39. F. Ramirez and J. F. Marecek, Tetrahedron 35, 1581 (1979).
40. A. Satterthwait and F. H. Westheimer, J. Am. Chem. Soc. 100, 3197 (1977).
41. F. Ramirez and J. F. Marecek, Tetrahedron, In Press.
42. M. K. Chantooni, Jr. and I. M. Kotthoff, J. Phys. Chem. 79, 1176 (1975).
43. K. Lohmann, Biochem. Z. 254, 381 (1932).
44. A. Hock and G. Huber, Biochem. Z 328, 44 (1956).
45. C. Liébecq and M. Jacquemotte-Louis, Bull. Soc. Chim. Biol. 40, 67, 759 (1958).
46. D. Lipkin, R. Markham and W. H. Cook, J. Am. Chem. Soc. 81, 6075 (1959).
47. S. L. Friess, J. Am. Chem. Soc. 75, 323 (1953).
48. M. Tetas and J. M. Lowenstein, Biochemistry 2, 350 (1963).
49. P. W. Schneider and H. Brintzinger, Helv. Chim. Acta 47, 1717 (1964).
50. D. H. Buisson and H. Sigel, Biochim. Biophys. Acta 343, 45 (1974).
51. M. M. Taqui Khan and M. Srinivas Mohan, J. Inorg. Nucl. Chem. 36, 707 (1974).
52. M. M. Taqui Khan and M. Srinivas Mohan, Indian J. Chem. 14A, 945, 951 (1976).
53. D. P. N. Satchell and R. S. Satchell, Annu. Rep. Prog. Chem., Sect. A 1978, 75, 41 (1979).
54. A. A. Frost and R. G. Pearson, Kinetics and Mechanism, 2nd ed., John Wiley & Sons, New York (1961).
55. D. L. Miller and F. H. Westheimer, J. Am. Chem. Soc. 88, 1507 (1966).
56. W. P. Jencks, Catalysis in Chemistry and Enzymology, McGraw Hill, New York (1969); pp 112-115.
57. T. C. Bruice and S. J. Benkovic, Bioorganic Mechanisms, W. A. Benjamin, New York (1966); Vol. 2, Ch. 7.
58. E. R. Stadtman, The Enzymes, 3rd ed., P. D. Boyer, Ed., New York, Academic Press (1973); Vol. VIII, Ch. 1.
59. R. L. Switzer, The Enzymes, 3rd ed., P. D. Boyer, Ed., Academic Press, New York (1974); Vol X, Ch. 19.
60. P. D. Boyer, Ed., The Enzymes, 3rd ed., Academic Press, New York (1973); Vol. VIII, IX.
61. G. Lowe, B. S. Sproat, J. C. S. Perkin I, 1622 (1978).
62. M. J. Cohn, J. Biol. Chem. 254, 2689 (1979).
63. I. A. Rose, Federation Proc. 37, 2775 (1978).
64. R. W. Lymn and E. W. Taylor, Biochemistry 10, 4617 (1971).
65. A. F. Huxley and R. M. Simmons, Nature, 233, 533 (1971).
66. C. G. Dos Remedios, R. G. C. Millikan and M. F. Morales, J. Gen. Physiol. 59, 103 (1972).
67. E. W. Taylor, Curr. Top. Bioenerg. 5, 201 (1973).
68. C. R. Bagshaw, J. F. Eccleston, F. Eckstein, R. S. Goody, H. Gutfreund and D. R. Trentham, Biochem. J. 141, 351 (1974).
69. M. Barany, K. Barany, C. T. Burt, T. Glonek and T. C. Myers, J. Supramolec. Struct. 3 125 (1975).
70. E. Eisenberg and T. L. Hill, Prog. Biophys. Mol. Biol. 33, 55 (1978).
71. T. Sekine and W. W. Kielley, Biochim. Biophys. Acta 81, 336 (1964).
72. M. Yamaguchi and T. Sekine, J. Biochemistry (Tokyo) 59, 24 (1966).
73. A. Mulhern and E. Eisenberg, Biochemistry 17, 4419 (1978).
74. W. F. Harrington, E. Reisler and M. Burke, J. Supramolec. Struct. 3, 112 (1975).
75. E. Reisler, M. Burke and W. F. Harrington, Biochemistry 13, 2014 (1974).
76. M. C. Schaub, J. F. Watterson, J. T. Walser and P. G. Waser, Biochemistry 17, 246 (1978).
77. M. Elzinga and J. H. Collins, Proc. Natn. Acad. Sci. U.S.A., 74, 4281 (1977).
78. R. Lu, J. Sosinki, M. Balint and F. Streter, Federation Proc. 37, 1965 (1978).
79. K. K. Shukla and H. M. Levy, Biochemistry 16, 5199 (1977).
80. K. K. Shukla and H. M. Levy, J. Biol. Chem. 253, 8362 (1978).
81. H. M. Levy and D. E. Koshland, Jr., J. Biol. Chem. 234, 1102 (1959).
82. C. R. Bagshaw, D. R. Trentham, R. G. Wolcott and P. D. Boyer, Proc. Nat. Acad. Sci. U.S.A. 72, 2592 (1975).
83. J. H. Young, J. McLick and E. F. Korman, Nature, 249, 474 (1974).